Interactions of Miltefosine and the Phospholipid DPPC at the air/water Interface

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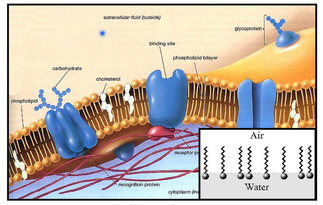
OBJECTIVE

Discovering the interaction and miscibility of the drug miltefosine and phospholipids of the cell membrane is important in determining the drugs mode of action which is still unknown. Langmuir monolayers serve as an ideal study model of the cell membrane lipid bilayer. Mixed monolayers of the phospholipid DPPC and miltefosine were created and analyzed. The behavior of the mixed monolayers was classified and related to a cell membrane to further understand miltefosine's mode of action.

BACKGROUD

Cell Membrane Lipid Bilayer vs. Monolayer:

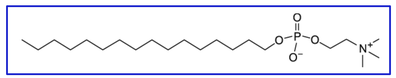


Cell membrane

The structural components of a biological membrane include lipids, proteins and carbohydrates. About 40% of the cell membrane is made up of three types of lipids: phospholipids, glycosphingolipids, and sterols.

The current study was designed to investigate miltefosine and the phospholipid DPPC. Phospholipids are essential components of cell membranes because of their role in regulating cell function, structural needs, and molecular recognition.

Miltefosine:

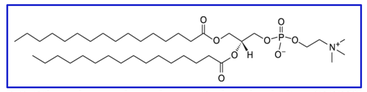


Hexadecylphosphocholine (Miltefosine)

Hexadecylphosphocholine (Miltefosine) is the first drug to have a phospholipid-like chemical structure. Recently, the drug has been a promising treatment for the protozoan parasite *Leishmania*.

Past studies investigated the interaction of miltefosine with cell membrane components: cholesterol, OPPE, and ganglipside GM1.

Phospholipid DPPC:

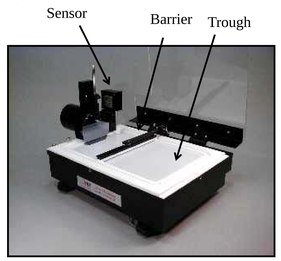


1,2-Dihexadecanoyl-sn-glycero-3-phosphocholine (DPPC)

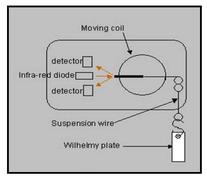
In this study, the interaction between miltefosine and particular components of the cell membrane continue to be investigated by examining the behavior of miltefosine and the phospholipid DPPC. The phospholipid DPPC is commonly used to model biological membranes.

Langmuir-Blodgett Trough:

A Nima Film Balance was used to detect changes in surface pressure of mixed monolayers spread on an air-water interface. The balance consists of a shallow Teflon trough, barrier, and sensor. The barrier is responsible for compressing the monolayer on the interface at a controlled speed. When compressed, the molecules at the surface are confined to a smaller surface area causing a monolayer to be formed.

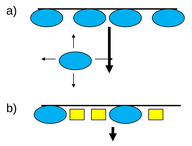


Langmuir-Blodgett Trough

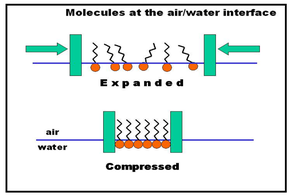


Sensor

Creating a Monolayer:



Surface tension a) pure water b) mixed solution



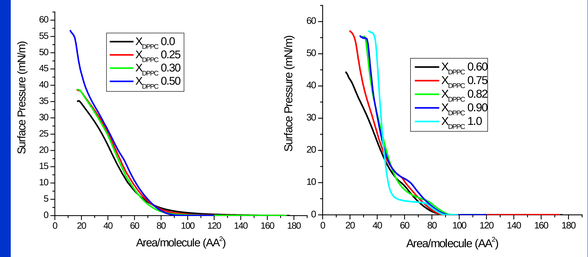
The molecules go through a series of specific phase changes characteristic of the molecule. As the monolayer forms, surface tension decreases. Pure water has a surface tension of approximately 72 mN/m, but a monolayer can cause this to drop nearly to zero (Nima, 2004). The change of surface tension is due to the same number of molecules being confined to a smaller amount of space. The change in surface tension is computed into a surface pressure reading by the sensor component of the instrument. The collected surface pressure readings create a surface-pressure area isotherm. A surface pressure of 30mN/m is similar to the environment found in a cell membrane.

METHODS

- 1:1 chloroform (Aldrich, 99+%) ethanol (Quantum Chemical Co., 200 proof) solvent
- Stock concentrations:
  - 0.498mg/mL Miltefosine (Avanti)
  - 0.75 mg/mL DPPC (Avanti)
- Mixed solutions X_{DPPC} :
  - 0.25, 0.30, 0.50, 0.75, 0.82, 0.90
- Experimental Settings:
  - Number of molecules: 3.43 x 10^{16}
  - Barrier speed: 50cm^2/min
- Data Analysis:
  - Surface-pressure area isotherms
  - Mean molecular area (A_{12}) values
  - Excess free energy of mixing (A_{exc}) values.

RESULTS

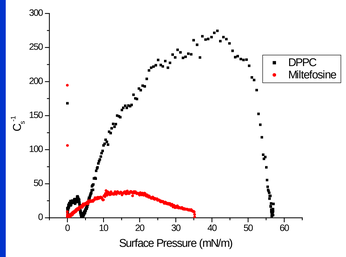
Surface Pressure Area Isotherm:



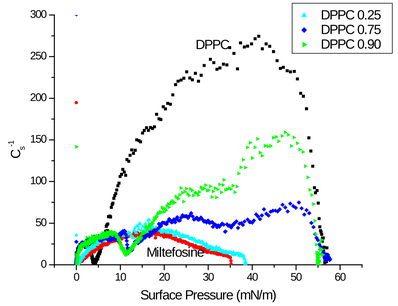
Above: Surface Pressure = initial surface tension – changing surface tension

Gas, liquid, and solid phase of a monolayer

Compression Modulus:

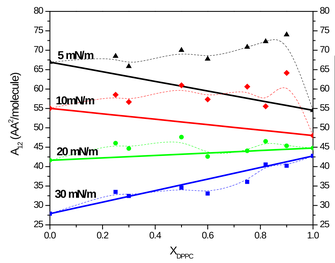


Range	Physical State
250 mN/m +	Solid
100-250 mN/m	Liquid Condensed
100 mN/m -	Liquid Expanded
Little/No Compression	Gas



Mean Molecular Area:

What type of interactions are observed in the mixtures?



- Solid line = ideal behavior
- Data points = actual area/molecule observed in mixture solutions
- Below the ideal = attractive forces
- Above the ideal = repulsive forces

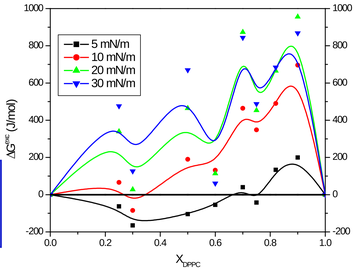
$A_{12} = A_1X_1 + A_2X_2$

Excess Free Energy:

How strong are the observed interactions?

- Magnitude of ΔG_{exc} value correlates to strength of interaction
- Positive = repulsive forces
- Negative = attractive forces

$\Delta G_{exc} = N\pi\int_0 A_{exc}\, d\pi$
$N = 6.023 \times 10^{23}$
(Avogadro's number)
$A_{exc} = A_{12} - A_1X_1 - A_2X_2$



CONCLUSIONS

- **Surface pressure(π)- area isotherms:** As the concentration of one of the components (DPPC) in the mixed monolayers increased, isotherms resembled the features displayed in the pure isotherm of the main component. Stability of the mixed monolayers increased substantially at X_{DPPC} 0.50 and higher concentrations. Collapse pressures changed with composition, which confirms that the monolayers are mixed vs. a two phase system.
- **Compression Modulus:** Pure DPPC monolayers have two phases: liquid expanded and solid. Pure miltefosine monolayers are in one phase: liquid expanded. Mixtures show characteristics of both components.
- **Mean molecular area vs. composition:** Low surface pressures show positive deviations from ideal behavior. Data at 30mN/m show both positive and negative deviations from ideal behavior.
- **Excess free energy of mixing vs. composition:** At increasing DPPC concentrations the excess free energy values become more positive. Low surface pressure, 5 mN/m, show negative and positive excess free energy values.
- **Overall:** Monolayers at 30mN/m models membranes. DPPC is likely to interact with miltefosine at the cell membrane based on the findings at 30mN/m.

REFERENCES

NIMA Technology, http://www.nima.co.uk/, 2005; Abel, E., et al, 2000.; Yau, W., et al, 1998.

mez-Serranillos, I. R., et al. "Study of the π-A Isotherms of Miltefosine Monolayers Spread at the air/water Interface." Physical Chemistry Chemical Physics 6.7 (2004): 1580-6. SCOPUS. Scopus. 14 June 2007.

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